

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

Screwworm Research Laboratory

P. O. Box 986

Mission, Texas 78572

May 23, 1973

Subject: Genetic Contamination of a New Breeding Stock

To: E. A. Taylor
Area Director
Subtropical Texas Area

Last year Dr. Dinah Childress, of our Fargo Laboratory, drew up a breeding scheme to incorporate maximum genetic variability in a new Texas screwworm stock. Under Dr. Crystal's direction, ARS technicians collected screwworms from 17 locations (15 counties) in Texas and added an 18th group from a new APHIS colony. Males from one locality were crossed with females from another in reciprocal crosses until all 18 collections were combined into one strain which Dr. Crystal designated "TX-New Texas," and I called the "18 Grandmas" strain.

The crossbreeding to establish one stock with this heredity took several generations, so the colony was not turned over to APHIS until November 1972. A small reference stock was retained in Dr. Crystal's laboratory. The flies were so "wild" in their behavior that they did not adapt readily to mass production, so field evaluation was delayed. APHIS is building a special adult colony room, 4 times larger than the old one, to accommodate this new stock for holding the flies uncrowded under day-night conditions. The new room will be ready in mid-June.

In the meantime, the Methods Development group of APHIS has been doing some comparative field studies with the new and the current production strain. Last week Dr. H. C. Hofmann, a Methods Development entomologist, told me that he had found 17 white-eyed flies in about 1100 adults of the 18-Grandma strain. I had our technician get all the dead flies from ARS cages for Dr. Hofmann to check. He found no genetic markers in more than 400 ARS flies of our portion of the strain.

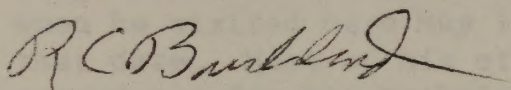
APHIS is discarding their contaminated stock to be replaced by a new one from ARS which will be a combination of the 18 Grandmas strain with a new Mexican strain which is a consolidation of 6 egg masses collected last October and November by APHIS inspectors in 3 states of Northern Mexico. Dr. Crystal reared the adults and combined them into a single strain. This strain is now being crossed: males of New Mexico X females of 18 Grandmas and the reciprocal to make one new 24 Grandmas strain. We

Genetic Contamination of New Breeding Stock

May 23, 1973²

expect to get about 200,000 eggs from this cross when the cages are egged on May 28. ARS will rear the larvae and give the pupae to A. J. Graham, Chief Entomologist of APHIS, who will personally supervise further rearing to prevent contamination. The new strain can be in massproduction for a critical field test by August.

In discussing contamination problems associated with the incident, Dr. M. E. Meadows, APHIS Director at Mission, suggested that ARS be responsible, not only for new genetic stocks but for all stocks in intermediate stages of development and that APHIS keep only a mass production colony. I said that I thout it would cost a million dollars a year for ARS to assume a responsibility that might be considered Methods Development rather than research. Dr. Meadows said that, if we wanted to take on the work, he would favor that expansion of our responsibility with the rearing being done in a separate building as our research stocks are now maintained.



R. C. Bushland
Research Leader

Dr. Crystal
RCB
File

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 23, 1973

Subject: Genetic Deterioration of Screwworm Stocks

To: E. A. Taylor
Area Director
Subtropical Texas Area

Dr. Bush of the University of Texas has done comparative enzyme studies on screwworm flies since April 20. He has not made a formal report, but he gave a most informative seminar and also told me more about his results when he visited here May 14 and 15 and in telephone conversations since that date. Most of his effort has been to develop standard analytical procedures for his technician to follow while he is in Europe this summer.

In this preliminary work, he has tested for 36 different enzyme systems and has found 20 that give positive readings against screwworm flies. He tried all 20 systems against the APHIS mass production stock and found that strain to be polymorphic for only one enzyme. He did not work as extensively with the ARS reference strains, but he did find more genetic variability. I am attaching a document Dr. Bush handed out at his seminar. It is entitled "Comparisons of Laboratory Strains" and summarizes results with 7 ARS strains compared against the APHIS regular production flies.

Dr. Bush told me over the telephone today of his results with flies reared from a "wild" egg mass collected locally three weeks ago by John Turner of our staff. Although these flies were siblings, the progeny of one wild female, they were polymorphic for 4 of 20 enzymes tested. There was more genetic variability in this one wild egg mass than in any of our laboratory colonies, indicating a great loss of heterozygosity through laboratory rearing. The extra bottleneck of mass rearing in the plant further reduces variability.

I think that our cooperative agreement with the University of Texas is already paying great dividends. Isoenzyme analysis will be a valuable tool to identify genetic variability in wild populations and to trace genetic deterioration in laboratory and plant production stocks. Enzyme studies will also aid us in assembling new breeding stocks of maximum genetic variability and maintaining "wild" heredity in mass production flies.

R. C. Bushland
R. C. Bushland
Research Leader

Enclosure

Comparisons Between Laboratory Strains*
(5 most polymorphic loci only)

Adults	LAP	ALD	PGM	GDH	EST
Regular Production					+
NTS Texas Strain				+	+
C3-F5 Texas Collection			+	+	+
C9-F1 Latest Mex. Coll.	+			+	+
APHIS, Old Mex. Strain		+		+	+
PRN-F9 Puerto Rico					+
TX-F8 New Texas Strain		+	+		+
RF Regular Florida				+	+

* All flies from Screwworm Research Laboratory, Mission, Texas.



THE UNIVERSITY OF TEXAS AT AUSTIN
AUSTIN, TEXAS 78712

Department of Zoology

19 JUN 1973

B 4 JUN 1973

File

May 29, 1973

Dr. Maxwell M. Crystal
Screwworm Research Laboratory
Mission, Texas 78572

Dear Dr. Crystal:

Enclosed you will find an outline of proposed research projects related to our study on screwworm population genetics. I think they are all self-explanatory. If you have any questions call my assistant, Ms. Mary Jane Andrews and she can possibly help you out.

I am leaving for Europe today and will return in late July. Best of luck.

Sincerely,

Guy L. Bush
Associate Professor

GLB:dj

UT - USDA Cooperation Screwworm Populations

Genetics Project May 30, 1973

Subject: Suggested research program for the genetic studies on
Screwworm Fly.

1. Selected crosses to establish pattern of inheritance of each polymorphic enzyme system. See the enclosed outline for the procedure to be followed for each individual cross. The strains to be used in crosses are as follows.

<u>STRAIN</u>	<u>PROTEIN(S)</u>
a) C3 Texas Collection	PGM, α GDH, EST
b) C9 Latest Mexican Collection	LAP, α GDH, EST
c) TX New Texas Strain	ALD, PGM, EST
d) Mission egg mass	MDH, PGM, α GDH, EST

2. Establish gene frequencies in all lab strains and those being developed for mass rearing.
 - a) Send at least 25-30 adults from each new lab strain started by ARS. This sample (preferably taken in the F_1) will be used as a base line for comparisons in all future samples of the same strain.
 - b) Send 25-30 individuals of each old lab strain not yet sampled.
 - c) At periodical intervals and at each major step from lab adaptation to mass rearing, samples of each strain should be sampled

and analyzed electrophoretically. This will give us some idea of what is happening at genetic level during colonization and mass rearing. From these results we should be able to develop a suitable genetic monitoring technique you can use later.

3. Continue sampling wild egg masses.

- a) Only single egg masses should be reared. Be sure that egg masses are not mixed.
- b) At least 15 adults from each egg mass should be sent.
- c) We need to sample about 30-50 parents from each area if we are to get good estimates of gene frequency. (i.e. 15-25 egg masses would provide frequency data on 30-50 parents.)

Preparation of Screwworms for Isozyme Studies

May 30, 1973

Adults and third instar larvae should be placed in the plastic screw-top vials provided, and frozen and held on dry ice following the procedure outlined below.

Adults can be anesthetized with CO₂ to facilitate handling and transfer to the plastic storage and shipping vials. No other chemicals should be used. Larvae can be placed in the vials directly without pretreatment.

Adults should be sexed and placed in separate vials. This greatly facilitates handling prior to electrophoresis, as it is sometimes difficult to recognize the sex of frozen specimens that have been damaged during shipment.

A loosely packed wad of cotton should be gently pushed into each vial and carefully pressed against the frozen specimens. This will keep the larvae and adults from breaking apart during shipment.

It is essential that each vial have an identification label with information on sex placed inside the vial, and another affixed to the outside with scotch tape. I will also need detailed background information on the nature of the collection, such as (1) locality, (2) date of collection, (3) altitude of collection site, (4) host animal, (5) collector's name, (6) number of generations in culture if applicable, (7) larval food used in culture. This information must be included on the labels provided.

The specimen vials can be stored on dry ice until enough specimens are accumulated for shipment. UNDER NO CIRCUMSTANCES SHOULD THE SPECIMENS BE ALLOWED TO THAW AT ANY TIME. If they are allowed to thaw, it will render the material useless for enzyme analysis.

Vials should be placed together in a strong plastic bag when ready for shipment. Just prior to shipment, they should be placed in the dry-ice-shipping-container, and the container filled with as much dry ice as possible. Care should be taken to use large pieces of dry ice, as small pieces tend to evaporate more rapidly.

The container should then be shipped by Grayhound Bus to:

Dr. Guy L. Bush
Department of Zoology
University of Texas
Austin, Texas 78712

Telephone (512) 471-5598

The time of departure of the container should be obtained prior to the preparation of a shipment. In this way the container will not have to sit around the bus depot, awaiting the departure of a bus. The container should hold ice for about 3-1/2 days so there is normally ample time for delivery.

As soon as the container has been shipped, I should be notified by phone of the time of departure, the bus number, estimated time of arrival, and the invoice number of the shipment. If the shipment is delayed, the information will permit me to trace the container before the dry ice is depleted.

Allozyme Inheritance Studies for Screwworm Flies

May 30, 1973

The following is an outline of the procedures to be followed in setting up and handling screwworm matings for allozyme inheritance studies. The objective of this study is to establish the mode of inheritance of the genetically polymorphic enzymatic proteins thus far discovered in our preliminary allozyme survey. Once the Mendelian characteristics of these allozymes have been established, they can be used as genetic markers for a wide range of studies on dispersal, ecology and population genetics of laboratory and natural populations.

Ten single-pair matings from each specified lab strain can be made in the following way:

A virgin female should be mated to one male, and her eggs collected and larvae reared separately. Great care should be taken not to mix progeny from different females. Each cross should be numbered consecutively by strain to facilitate handling and for identification purposes.

The male and female parents of each cross should be frozen, and placed together in a separate, clearly labeled plastic vial. These should be given the same cross-number as their progeny and prefixed with the letter P (P stands for parental). As an example, the label should read Cross P-20. The progeny of this cross should be labeled F-20 (the F stands for filial).

The broods should be permitted to pupate and the adults collected within 6-7 days after emergence. The adults should then be sexed, and all individuals from a single brood and of the same sex placed together in a plastic vial. The vials with the parents and progeny should be taped together to avoid confusion when processing.

With the parents and their progeny available for analysis, it will be possible to examine the frequencies of each allele resulting from the cross and compare the observed with the expected values. It is then a simple matter to establish whether the alleles in question are inherited in a simple Mendelian fashion.

At no time should any of this material be permitted to thaw and, if possible, the adults and larvae should be maintained on dry ice until ready for shipment.

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 23, 1973

Subject: Genetic Contamination of a New Breeding Stock

To: E. A. Taylor
Area Director
Subtropical Texas Area

Last year Dr. Dinah Childress, of our Fargo Laboratory, drew up a breeding scheme to incorporate maximum genetic variability in a new Texas screwworm stock. Under Dr. Crystal's direction, ARS technicians collected screwworms from 17 locations (15 counties) in Texas and added an 18th group from a new APHIS colony. Males from one locality were crossed with females from another in reciprocal crosses until all 18 collections were combined into one strain which Dr. Crystal designated "TX-New Texas," and I called the "18 Grandmas" strain.

The crossbreeding to establish one stock with this heredity took several generations, so the colony was not turned over to APHIS until November 1972. A small reference stock was retained in Dr. Crystal's laboratory. The flies were so "wild" in their behavior that they did not adapt readily to mass production, so field evaluation was delayed. APHIS is building a special adult colony room, 4 times larger than the old one, to accommodate this new stock for holding the flies uncrowded under day-night conditions. The new room will be ready in mid-June.

In the meantime, the Methods Development group of APHIS has been doing some comparative field studies with the new and the current production strain. Last week Dr. H. C. Hofmann, a Methods Development entomologist, told me that he had found 17 white-eyed flies in about 1100 adults of the 18-Grandma strain. I had our technician get all the dead flies from ARS cages for Dr. Hofmann to check. He found no genetic markers in more than 400 ARS flies of our portion of the strain.

APHIS is discarding their contaminated stock to be replaced by a new one from ARS which will be a combination of the 18 Grandmas strain with a new Mexican strain which is a consolidation of 6 egg masses collected last October and November by APHIS inspectors in 3 states of Northern Mexico. Dr. Crystal reared the adults and combined them into a single strain. This strain is now being crossed: males of New Mexico X females of 18 Grandmas and the reciprocal to make one new 24 Grandmas strain. We

Department of Agriculture
P. O. Box 282
Mission, Texas 75702

May 22, 1952

Subject: Genetic Contamination of a New Breeding Stock

To: E. A. Taylor
Area Director
Subtropical Texas Area

Last year Dr. Daniel Childress, of our Fargo Laboratory, drew up a breeding scheme to incorporate maximum genetic variability in a new Texas sorghum strain. Under Dr. Childress' direction, ARS technicians collected sorghum from 12 locations (12 counties) in Texas and added an 18th group from the APHIS colony. Males from one locality were crossed with females from another in reciprocal crosses until all 18 collections were combined into one strain which Dr. Childress designated "TX-New Texas", and I called the "Grainman" strain.

The crossbreeding to establish one stock with this heredity took several generations. The new colony was not turned over to APHIS until November, 1951. A small reference stock was retained in Dr. Childress' laboratory. The files were so "wild" in their behavior that they did not adapt readily to mass production, so field evaluation was delayed. APHIS is building a special APHIS colony room, a times larger than the old one, to accommodate this new stock for holding the files under better day-night conditions. The new room will be ready in mid-June.

In the meantime, the Methods Development group of APHIS has been doing some comparative field studies with the new and the current APHIS strain. Last week Dr. E. C. Hoffman, a Methods Development entomologist, told me that he had found 17 whitefly infestations in about 100 acres of the 18-Grainman strain. I had not noticed any but all the 18-Grainman from ARS came from Dr. Hoffman's stock. He found no genetic markers in more than 500 ARS lines of our section of the strain.

APHIS is also doing field contamination studies to be reported by a new one from ARS which will be a combination of the 18-Grainman strain with a new Mexican strain which is a combination of a very narrow selected line. Dr. Childress and I are also studying the 18-Grainman strain in a large amount. This strain is now being crossed with a new strain of 18-Grainman. The 18-Grainman and the reference to a new 18-Grainman strain.

Genetic Contamination of New Breeding Stock

May 23, 1973²

expect to get about 200,000 eggs from this cross when the cages are egged on May 28. ARS will rear the larvae and give the pupae to A. J. Graham, Chief Entomologist of APHIS, who will personally supervise further rearing to prevent contamination. The new strain can be in massproduction for a critical field test by August.

In discussing contamination problems associated with the incident, Dr. M. E. Meadows, APHIS Director at Mission, suggested that ARS be responsible, not only for new genetic stocks but for all stocks in intermediate stages of development and that APHIS keep only a mass production colony. I said that I thout it would cost a million dollars a year for ARS to assume a responsibility that might be considered Methods Development rather than research. Dr. Meadows said that, if we wanted to take on the work, he would favor that expansion of our responsibility with the rearing being done in a separate building as our research stocks are now maintained.

R. C. Bushland
Research Leader

May 23, 1973

Genetic Contamination of New Breeding Stock

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R. C. Bushland
Research Leader

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P. O. Box 986
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May 23, 1973

Subject: Genetic Contamination of a New Breeding Stock

To: E. A. Taylor
Area Director
Subtropical Texas Area

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The crossbreeding to establish one stock with this heredity took several generations, so the colony was not turned over to APHIS until November 1972. A small reference stock was retained in Dr. Crystal's laboratory. The flies were so "wild" in their behavior that they did not adapt readily to mass production, so field evaluation was delayed. APHIS is building a special adult colony room, 4 times larger than the old one, to accommodate this new stock for holding the flies uncrowded under day-night conditions. The new room will be ready in mid-June.

In the meantime, the Methods Development group of APHIS has been doing some comparative field studies with the new and the current production strain. Last week Dr. H. C. Hofmann, a Methods Development entomologist, told me that he had found 17 white-eyed flies in about 1100 adults of the 18-Grandma strain. I had our technician get all the dead flies from ARS cages for Dr. Hofmann to check. He found no genetic markers in more than 400 ARS flies of our portion of the strain.

APHIS is discarding their contaminated stock to be replaced by a new one from ARS which will be a combination of the 18 Grandmas strain with a new Mexican strain which is a consolidation of 6 egg masses collected last October and November by APHIS inspectors in 3 states of Northern Mexico. Dr. Crystal reared the adults and combined them into a single strain. This strain is now being crossed: males of New Mexico X females of 18 Grandmas and the reciprocal to make one new 24 Grandmas strain. We

Seaworm Research Laboratory
P. O. Box 986
Mission, Texas 78147

May 22, 1973

Subject: Genetic Composition of a New Breeding Stock

To: E. A. Taylor
Area Director
Subtropical Texas Area

Last year Dr. Brian Chittress, of our Fargo Laboratory, grew up a breeding stock to incorporate maximum genetic variability in a new Texas seaworm stock. Under Dr. Crystal's direction, ARS technicians collected seaworms from 15 locations (12 counties) in Texas and added an ARS group from a new ARS colony. Males from one locality were crossed with females from another in reciprocal crosses with all 15 collections were combined into one strain which Dr. Crystal designated "TX-New Texas" and I called the "18 Grandmas" strain.

The crossbreeding to establish one stock with this heredity took several generations, so the colony was not turned over to RHIS until November, 1972. A small reference stock was retained in Dr. Crystal's laboratory. The lines were so "wild" in their behavior that they did not breed readily to waste production, so final evaluation was delayed. ARHS is holding a special adult colony room, a times larger than the old one, to accommodate this new stock for holding the lines unmated under day-night conditions. The new room will be ready in mid-June.

In the meantime, the Methods Development group at ARHS has been doing some comparative field studies with the new and the current production strain. Last week Dr. H. C. Hottelmann, a Methods Development technician, told me that he had found 17 white-eyed flies in about 100 adults of the 18-Grandmas strain. I had our technician get all the dead flies from ARS cages for Dr. Hottelmann to check. He found no genetic markers in more than 400 ARS flies of any portion of the strain.

ARHS is expecting their commercial stock to be replaced by a new one from ARS which will be a combination of the 18-Grandmas strain with a new Mexican strain which is a combination of 8 egg masses collected last October and November by ARHS inspectors in 3 states of Northern Mexico. Dr. Crystal feared the adults and combined them into a single strain. This strain is now being crossed; males of New Mexico & females of 18-Grandmas and the reciprocal to make one new 26-Grandmas strain. We

expect to get about 200,000 eggs from this cross when the cages are egged on May 28. ARS will rear the larvae and give the pupae to A. J. Graham, Chief Entomologist of APHIS, who will personally supervise further rearing to prevent contamination. The new strain can be in massproduction for a critical field test by August.

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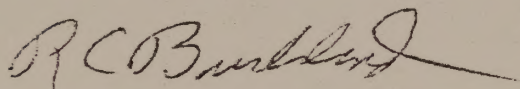
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R. C. Bushland
Research Leader

GENETIC LOCI OF THE SCREW-WORM FLY
(Laboratory Strains Only)

May, 1973

TYPE	LOCUS	NAME	ADULTS	LARVAE	NO. ALLELES
<u>Protein</u>					
	P	Protein A	+	?	1
<u>Hydrolases and Lyases</u>					
	Amy	Amylase	+	+	1
	ACPH	Acid phosphate	+	0	1
	ALD-A	Aldolase-A	+	+	2
	ALD-B	Aldolase-B	0	+	1
	ALD-C	Aldolase-C	0	+	1
	APH-A	Alkaline phosphatase-A	+	+	1
	APH-B	Alkaline phosphatase-B	+	+	1
	β EST-A	β Esterase-A	+	0	4+
	α EST-B	α Esterase-B	+	0	2
	EST-C	Esterase-C	0	+	2+
	EST-D	Esterase-D	0	+	3+
	LAP-A	Leucine amino peptidase-A	0	+	1
	LAP-B	Leucine amino peptidase-B	+	+	2(?)
<u>Dehydrogenases</u>					
	ADH-A (HDL)	Alcohol dehydrogenase-A	+	+	1
	ADH-B (HDL)	Alcohol dehydrogenase-B	+	+	1
	α -GPD	α -Glycerophosphate	+	?	3
	IDH	Isocitrate dehydrogenase	+	+	1
	LDH	Lactate dehydrogenase			
	MDH-D	Malate dehydrogenase DPN dependent	+	?	1
	MDH-TA	Malate dehydrogenase TPN dependent-A	0	+	1
	MDA-TB	Malate dehydrogenase TPN dependent-B	+	+	1
	6-PGD	6-Phosphogluconic dehydrogenase	+	+	1
	G6PD	Glucose-6-phosphate	+	+	1
	SORB	Sorbital dehydrogenase	0	?	
	GDH	Glutamate dehydrogenase	+	?	1
	G3PD	Glyceraldehyde-3-phosphate	+	0	1
	GalDH	Galactose dehydrogenase	0	?	?
	Pep	Pepsinogen			

GENETIC LOCI OF THE SCREW-WORM FLY
(Laboratory Strains Only)
May 1973

TYPE	LOCUS	NAME	ADULTS	LARVAE	NO. ALLELES
<u>Dehydrogenase Coupled Enzymes</u>					
	AK	Adenlate kinase	+	?	?
	Hex	Hexokinase	+	0	1
	PGI-A	Phosphoglucose isomerase-A	+	+	1
	PGI-B	Phosphoglucose isomerase-B	0	+	?
	PGM	Phosphoglucose mutase	+	+	3
	FUM	Fumarase	+	+	1
<u>Miscellaneous Enzymes</u>					
	GOT-A	Glutamate-oxaloacetate transaminase-A	0	+	1
	GOT-B	Glutamate-oxaloacetate transaminase-B	+	+	1
	GOT-C	Glutamate-oxaloacetate transaminase-C	0	+	2(?)
	Tyr	Tyrosinase	1 or 0	?	1
	Al	Aldehyde oxidase	+	0	1
	To	Tetrazolium oxidase	+	0	1

Total number of loci examined = 36

Polymorphic in adults = 21.4% (6/28)

Polymorphic in larvae = 25% (6/24)

Polymorphic - all loci = 25% (9/36)

Preliminary isoenzyme analysis conducted by:

Dr. Guy L. Bush
Associate Professor of Zoology
Department of Zoology
University of Texas
Austin, Texas 78712

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 23, 1973

Subject: Genetic Deterioration of Screwworm Stocks

To: E. A. Taylor
Area Director
Subtropical Texas Area

Dr. Bush of the University of Texas has done comparative enzyme studies on screwworm flies since April 20. He has not made a formal report, but he gave a most informative seminar and also told me more about his results when he visited here May 14 and 15 and in telephone conversations since that date. Most of his effort has been to develop standard analytical procedures for his technician to follow while he is in Europe this summer.

In this preliminary work, he has tested for 36 different enzyme systems and has found 20 that give positive readings against screwworm flies. He tried all 20 systems against the APHIS mass production stock and found that strain to be polymorphic for only one enzyme. He did not work as extensively with the ARS reference strains, but he did find more genetic variability. I am attaching a document Dr. Bush handed out at his seminar. It is entitled "Comparisons of Laboratory Strains" and summarizes results with 7 ARS strains compared against the APHIS regular production flies.

Dr. Bush told me over the telephone today of his results with flies reared from a "wild" egg mass collected locally three weeks ago by John Turner of our staff. Although these flies were siblings, the progeny of one wild female, they were polymorphic for 4 of 20 enzymes tested. There was more genetic variability in this one wild egg mass than in any of our laboratory colonies, indicating a great loss of heterozygosity through laboratory rearing. The extra bottleneck of mass rearing in the plant further reduces variability.

I think that our cooperative agreement with the University of Texas is already paying great dividends. Isoenzyme analysis will be a valuable tool to identify genetic variability in wild populations and to trace genetic deterioration in laboratory and plant production stocks. Enzyme studies will also aid us in assembling new breeding stocks of maximum genetic variability and maintaining "wild" heredity in mass production flies.

R. C. Bushland
Research Leader

Enclosure

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 23, 1973

Subject: Genetic Deterioration of Screwworm Stocks

To: E. A. Taylor
Area Director
Subtropical Texas Area

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Enclosure

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NATIONAL PROGRAM STAFF
Plant and Entomological Sciences
Beltsville, Maryland 20705

89 AUG 1973

August 27, 1973

Subject: Genetic Mechanisms for Insect Control

To: All Research Stations for Insects Affecting Man, Animals,
and Stored Products

Within the last few days a manuscript was circulated to me from NTE that caught my attention. I hasten to say this is not the only manuscript that has impressed me, and that this memo does not reflect partiality or favoritism. I congratulate all of you on the fine flow of manuscripts reporting on a wide range of excellent research. I urge you to keep it up for the benefit of the specific areas of entomological research that are of direct concern to us. The reason for attention to this particular manuscript is to bring to your attention information that may be helpful in your various research programs.

The manuscript is by Dr. LaBrecque of Gainesville and the title is, "Genetic Control of Mosquitoes." It covers a paper presented at the International Symposium on Mosquito Control at Three Rivers, Quebec, Canada, May 8-10, 1973. The paper will presumably appear in the proceedings of the symposium.

Although the subject is addressed to mosquito control, it has much broader potential application. The paper is of a review nature and includes 32 literature citations. It discusses nine types of genetic mechanisms for insect control, the first five of which have been tried against mosquitoes, the remaining four remain to be tried. The mechanisms are as follows:

1. Dominant lethality.
2. Deleterious genes.
3. Cytoplasmic incompatibility.
4. Hybrid sterility.
5. Translocations.
6. Aspermia.
7. Sperm inactivation.
8. Meiotic drive.
9. Compound chromosomes.

The matter of genetic control may not be of concern to all of you. To those who are interested, I would suggest you watch for the publication of the article or request a reprint or copy of the manuscript from Dr. LaBrecque.

L. S. Henderson

L. S. Henderson
Staff Scientist

cc:
Dr. LaBrecque
Dr. Klassen



THE UNIVERSITY OF TEXAS AT AUSTIN

AUSTIN, TEXAS 78712

Department of Zoology

August 30, 1973

Dr. R. C. Bushland
Screwworm Research Laboratory
P.O. Box 986
Mission, Texas 78572

Dear Bush,

I enjoyed our brief visit to the Mission lab and the discussion of our screw worm genetic study was most profitable. I was particularly pleased to see Ed Taylor once again. As a result of our conversation on financing the project, Bob Flake and I have prepared a brief summary on how we plan to handle the genetic data from a statistical standpoint.

As I feel that the contributions of Dr. Flake and his graduate student are essential to the success of our genetic program. I will go ahead and pay his graduate student part time stipend for the fall semester. This amounts to \$1,400. This will allow him to start on the project immediately. I hope that this amount can be replaced by the small supplementary grant requested.

As soon as we have the analysis of the laboratory and production strains completed I will send you the results.

Very best regards,

A handwritten signature in dark ink, appearing to be "J. M. Smith", written in a cursive style.

Justification of Suplimentary Fund Request.

Preliminary analysis of a number of screwworm strains has established that electrophoretic data are a useful indicator of the genetic changes occurring during the adaptation of screwworms to current mass-rearing conditions. These data also indicate that the mass colonization process has the effect of greatly reducing genetic variability. Such losses which could also reduce the mating success of the flies in nature is undoubtedly the result of intense selection and inbreeding within the production strains.

If a realistic assessment of genetic variation is to be obtained appropriate statistical methods for treating population data must be applied. Such methods have been developed by Dr. Flake over the past five years utilizing chemosystematic characters in plants. It is proposed to modify these techniques to deal with discrete genotypic information of the type obtained by electrophoresis and incorporate an appropriate measure of genetic distance between populations. Several such measures have been proposed recently. Reprints of the most pertinent references on genetic distance and Dr. Flake's populational studies are enclosed.

The statistical methods to be developed for the analysis of genetic structure will be employed to estimate the amount and rate of genetic change that is apparently occurring during the adaptation of wild screwworms to the mass-rearing facility. The results of the study could be incorporated into a quality control program with the goal of monitoring and maintaining a desired level of genetic variability and thus insuring against the lose of competitive behavior traits which may be under genetic control.

Similar statistical techniques can also be employed to analyze the geographic structure of natural populations in order to detect the presence of possibly distinct geographical races. The knowledge of the existence of geographical races is an important factor to consider in designing future sampling programs and for establishing new production strains.

Dr. Flake has agreed to undertake the modification of his statistical technique as indicated above and to apply these methods in collaboration with me to the screwworm studies now underway. Partial support for salaries of a graduate student and partial summer salary for himself are requested. The part-time summer salary will free Dr. Flake from teaching responsibilities so he can devote full time to this project. Travel funds are also requested to cover transportation of Dr. Flake and his student for trips to Mission and to Mexico.

The breakdown of the budget for one year of support is as follows:

Salaries and wages

(Ph.D. Student 1/2 time 12 mo.)	\$4200	
(Dr. Flake 1 1/2 mo. summer)	2600	
	<u>\$6800</u>	
(Fringe benefits)	625	
	<u>\$7425</u>	\$7425
(computer time & computer supplies)		275
(travel to Mission and Mexico)		800
		<u>\$8500</u>

UT - USDA JOINT SCREWORM POPULATION GENETICS

PROJECT

Locality _____ Altitude _____
 Date collected ____/____/____ Egg mass Larvae Adults
 (circle one)
 Date frozen ____/____/____ Host animal _____
 Generations in lab ____ Lab. food _____
 Collectors name _____
 Remarks _____

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ACH 8pgs.

Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura*

1. RUDIMENTS OF SEXUAL ISOLATION*

By LEE EHRMAN

The Rockefeller Institute, New York City 21, U.S.A.

(Received 11 October 1963)

1. INTRODUCTION AND MATERIALS

In a series of studies published by M. Vetukhiv (1953, 1954*a* and *b*, 1956*a*, *b* and *c*, 1957; Vetukhiv & Beardmore, 1959; Wallace and Vetukhiv, 1955), he described the longevity, fecundity, and larval competitive ability of F_1 and F_2 hybrids between geographic populations of certain species of *Drosophila*. In most, though not in all cases, the F_1 hybrids were superior to the parental populations in all the parameters examined. In fact, the hybrid populations usually exceeded not only the mid-parent but even the superior parent. Contrasting with this, the F_2 hybrid generation suffers a 'hybrid breakdown'; not only does the F_2 lack the apparent heterosis of the F_1 hybrids but the second generation hybrids tend to fall below the parental populations. These results cannot be accounted for by supposing that the parents crossed were inbred; although the original strains used were kept in laboratory cultures for some generations before the beginning of the experiments, the 'parents' were never single strains but populations obtained by intercrossing all the available strains from a given locality. In other words, care was taken to obtain, in laboratory cultures, as faithful replicas of the natural populations as practicable. It appeared, then, that when the gene pools of two populations diverge, there are likely to arise, in some but not in all of them, genetic systems of a kind which produce heterosis in F_1 hybrids and a genetic breakdown in F_2 .

In May 1958, Dr Vetukhiv started a new series of experiments, designed to test the possibility that genetic systems of the above sort may arise not only in natural but also in experimental populations in the laboratory. He arranged six populations, to be kept in the wooden 'population cages' of a model which was widely used in the laboratory of Professor Th. Dobzhansky at Columbia University (Dobzhansky, 1947). Two of these populations were maintained in a constant temperature room at 16° C., two in an incubator or in a constant temperature room at 25° C., and the remaining two in an incubator at 27° C. The founders of these six populations were the same. In order to provide the populations with as much genetic variability as possible, to give selection material to work with, Vetukhiv chose to use as founders

* Work supported in part under Contract AT-(30-1)-3096, U.S. Atomic Energy Commission.

hybrids from more than forty strains, at least ten from each of the following geographic localities: Mather, California; Bryce National Park, Utah; Ferron, Utah; and Gunnison, Colorado. Each strain was the progeny of a single female, fertilized in nature by presumably a single male, collected in the respective localities by Professor Th. Dobzhansky in the summer of 1950. All strains were examined cytologically by Professor Dobzhansky, and all strains used by Dr Vetukhiv had the Arrowhead gene arrangement in their third chromosomes, and no other chromosomal polymorphisms. The technique of obtaining hybrids was as follows: virgin females from all the strains from the first locality were mated to males from all the strains from the second, and females from the third to males from the fourth, and vice versa (i.e. reciprocal crosses were made to insure equal participation of the gene pools of the four geographic populations). The F_1 hybrids were intercrossed to obtain quadruple hybrids. All these crosses were made in regular culture bottles. When the quadruple hybrids hatched, a group of about 1000 of them, about equally females and males, and derived about equally from all cultures, were placed in a population cage to serve as founders. The cups with food and with eggs deposited on them over a period of a day or two were removed to a population cage without adult flies. Fresh cups were introduced into the cage with the founders, and a new batch of eggs collected. This was repeated six times, so that six population cages were obtained with progenies of the same group of founders. The population cages were then distributed in pairs to the three different temperatures.

After the untimely death of Dr Vetukhiv in June of 1959, the populations were maintained by Mrs O. Pavlovsky, Mrs N. Spassky and, finally, by Mr B. Spassky. The temperature of 27°C . is close to the upper limit at which most strains of *D. pseudoobscura* can be kept in the laboratory for more than a single generation. The population cages at this temperature were at first difficult to maintain, and they had to be given temporary respite by transferring them to 25°C . for a generation or so. Eventually they became reasonably vigorous, enough to be maintained exclusively at 27°C ., although the numbers of adult flies in these populations were always smaller than in those kept at lower temperatures.

It is difficult to estimate how many generations intervened between the foundation of the populations and the time when flies were taken from them for the purposes of the experiments described below, except that at 16°C . there were only about half as many generations as at 25°C ., and at 25°C ., somewhat fewer than at 27°C .. It is probably fair to say that in population cages at 25°C ., there occur between ten and fifteen generations per year, and that the generations broadly overlap. (See Barker, 1962, for a discussion of the estimation of generation interval in experimental populations of *Drosophila*.)

2. METHOD

On October 6, 1962, when the six population cages were 4 years and 5 months old, samples of about twenty adults were taken from each cage and thereafter subcultured and maintained in uncrowded culture bottles under optimal nutritional

conditions at room temperature. The flies used for these experiments have, accordingly, developed under similar conditions. However, the six population cages and the temperature at which they existed for the 4 years and 5 months before the start of these experiments were:

A and B at 16° C.

C and D at 25° C.

E and F at 27° C.

Virgins of both sexes were collected from cultures of each line, and aged for 4 days at room temperature. (Males were sometimes aged for as long as 5 days.) Flies which were utilized in these experiments were etherized only once—when they were 'sexed'. At this same time, one wing of each of half of the females was clipped slightly on its distal margin for ease of identification. Groups of ten males of one kind with ten females of the same kind, plus ten more females of another cage sample ($10X\sigma\sigma + 10X\varphi\varphi + 10Y\varphi\varphi$), were then confined in vials containing food for 2 hours at 27° C. or for $2\frac{1}{4}$ hours at 16° C., the two extreme temperatures at which the population cages were kept. A pilot experiment has shown that these time intervals give about 50% of the females inseminated.

After 2 hours or $2\frac{1}{4}$ hours had elapsed, the entire contents of each vial were etherized and males discarded. Thus, males were never used more than once. The females were then sorted (clipped and non-clipped wings). Then the sperm-storing organs (ventral receptacles and spermathecae) were dissected out in physiological saline and examined for the presence of spermatozoa. The tallies from the following vials were discarded:

- (1) those in which fewer than twenty females were alive at the close of the multiple choice experiment,
- (2) those in which more than fourteen females (70%) were inseminated, and
- (3) those in which fewer than six females (30%) were inseminated.

Too little insemination indicates that the flies were apparently not in optimal condition when placed together. Too much insemination must be eliminated since the experimental scheme does not allow for the scoring of sequence of insemination.

With six populations involved, and taking into consideration two extreme temperatures and reciprocal crosses,

e.g. $10X\sigma\sigma + 10X\varphi\varphi + 10Y\varphi\varphi$ and $10Y\sigma\sigma + 10Y\varphi\varphi + 10X\varphi\varphi$,

twenty-four crucial crosses were tested. Two hundred females were dissected from each cross—one hundred of each of two kinds, making up ten vials, so that a total of 4800 females were dissected: $10 \times 10 \times 2$ (or 200) $\times 24 = 4800$. Of course, more than this number were actually dissected, but some counts had to be excluded from the data (see above).

No females from any one cage sample were consistently clipped. This was rotated so that in any given multiple choice cross, females from a single population were clipped only half the time. These experiments were begun on October 6, 1962, and concluded on May 3, 1963.

3. RESULTS

The data are summarized in Table 1. The isolation indexes were calculated according to Stalker (1942), because the per cent of females inseminated was close to fifty, or was exactly 50% in all instances.

Table 1. Percentages of females inseminated in different crosses

Populations Crossed	°C	Homogamic	Heterogamic	Isolation Index	Chi square
A × B	16	56	41	0.155	3.92*
	27	53	35	0.204	5.86**
B × A	16	53	49	0.039	0.18
	27	68	44	0.214	10.74**
C × D	16	47	47	0	0
	27	63	48	0.135	3.97*
D × C	16	62	43	0.181	6.50**
	27	61	37	0.245	10.58**
E × F	16	49	50	-0.010	0
	27	65	43	0.203	8.87**
F × E	16	54	43	0.113	2.00
	27	54	53	0.009	0
A × C	16	55	47	0.078	0.98
	27	60	58	0.017	0.02
C × A	16	51	44	0.073	0.72
	27	50	49	0.010	0
A × E	16	52	39	0.143	2.90
	27	48	41	0.078	0.73
E × A	16	60	43	0.165	5.12**
	27	52	57	-0.045	0.32
C × E	16	72	66	0.043	0.58
	27	69	42	0.243	13.68**
E × C	16	45	47	-0.022	0.02
	27	43	47	-0.033	0.18

* = Significant at the 5% level; ** = significant at the 2.5% level or better.

A Stalker isolation index of +1.00 means only homogamic matings, 0 means that only random matings are taking place, and -1.00 would mean exclusive occurrence of heterogamic matings. There were four only slightly negative isolation indexes but *all* twenty-four joint isolation indexes (simply the mean of the two reciprocal isolation indices) were positive (Table 2). For an excellent review of the value of these, and of other statistics, see Levene (1949).

The chi-squares were computed from two-by-two contingency tables with Yates' correction; each one has one degree of freedom. These are equivalent to a test for the significance of an isolation index. No test of significance for the joint isolation index has been devised.

In Table 1, the per cent inseminated is exactly equal to the number inseminated because in each cross (the rows) there were one hundred possibilities for homogamic

mating as well as one hundred possibilities for heterogamic mating. Thus, in the first cross in Table 1, $A \times B$ at 16°C ., 56 A females were inseminated and 44 were not; 41 B females were inseminated, while 59 were not.

Table 2. *Joint isolation indexes from different crosses*

Populations crossed	$^{\circ}\text{C}$	Index
$A \times B$	16	+0.097
	27	+0.209
$C \times D$	16	+0.090
	27	+0.190
$E \times F$	16	+0.052
	27	+0.106
$A \times C$	16	+0.075
	27	+0.014
$A \times E$	16	+0.154
	27	+0.016
$C \times E$	16	+0.011
	27	+0.105

The population indicated first, e.g. A in $A \times B$, denotes the type of male used. Thus, in $A \times B$, $A\sigma\sigma$ were confined with $A\phi\phi$ and $B\phi\phi$. The third row represents the cross reciprocal to the first, the fourth is reciprocal to the second, etc.

Nine of the chi-squares in Table 1 are significant at, at least, the 5% level; of the twenty-four crosses, nineteen show a greater number of homogamic than of heterogamic matings, four show a greater number of heterogamic matings, and in one cross the number was exactly equal.

4. DISCUSSION

Vetukhiv's (1954a) general conclusion from his experiments has been that 'the gene pool of the population of any one geographic region contains ... a variety of coadapted gene complexes ... Natural selection does not, however, adjust the gene complexes in different geographic populations ... for the simple reason that interbreeding of members of geographically remote populations occurs only rarely or not at all ... the genotype of each local population is, in at least some sexually reproducing species, an integrated system which may break down as a result of gene recombination in the hybrids.' How generally valid this conclusion may prove to be is at present an open question. McFarquhar & Robertson (1963) working with geographic races of *Drosophila subobscura* were unable to find any sign either of increased vigor of F_1 hybrids, or of a breakdown in the F_2 hybrids between populations from remote localities.

It is, therefore, especially interesting that, in *D. pseudoobscura*, experimental populations can diverge genetically within only few years time to a point where at least some of them show traces of sexual isolation, and, as will be described in a forthcoming paper by Dr A. Mourad, also of F_1 hybrid vigor and F_2 breakdown. The six experimental populations started by Dr Vetukhiv in 1958 were initially genetically similar, except for the possible differences introduced by sampling; these differences could not have been large because the number of the founders of each population was substantial, close to a thousand. Since the populations were then kept quite separate, with no exchange of migrants between them, the sexual isolation which has developed between some of these populations could not have been a result of selection specifically for such an isolation. It is more tempting to suppose that the sexual isolation arose as a by-product of genetic changes in populations which became adapted to different environments, especially to different temperatures (see the section on the origin of reproductive isolation in Ehrman, 1962). Even this view meets with difficulties; as an inspection of Tables 1 and 2 shows, traces of sexual isolation have appeared, if anything, more often between populations which were kept at the same temperature (populations A and B, C and D) than between populations which lived at different temperatures (no isolation at all between A kept at 16° and C kept at 25° , only a single instance between A and E and between C and E, E being the population kept at 27°).

Genetic divergence evidently takes place between isolated populations kept in similar as well as in different environments (it would, perhaps, be more correct to say in similar or in different macro-environments). Such divergence, due to the combined effects of genetic drift and natural selection, was demonstrated in experimental populations of *D. pseudoobscura* by Dobzhansky & Pavlovsky (1957) and Dobzhansky & Spassky (1962). When populations become different in more and more and more genes, reproductive isolation may arise because the action of many genes is pleiotropic. Some gene differences selected for different reasons, or resulting from random genetic drift, may thus have isolating side effects. These, to be sure, are mere rudiments of reproductive, in our case, sexual isolation. For the completion of the process of isolation, to the point where no hybrids at all would be formed between members of different populations, occasional gene exchange and production of adaptively inferior hybrids would probably be necessary stimuli (Dobzhansky, 1958).

In this connection, comparison of our results with those of Thoday & Gibson (1962) is of special interest. These investigators practiced diversifying (disruptive) selection for high and low sternopleural chaeta numbers on a single population of *D. melanogaster*. As a result of this selection, the population tended to split into two moieties, which, after as few as twelve generations of selection, produced only a limited number of hybrids. Unlike our experiments, in which the populations were kept quite separate, Thoday and Gibson's work seems to demonstrate the origin of reproductive isolation without an antecedent geographical separation. It is nevertheless clear that, as Thoday and Gibson admit, geographical separation facilitates greatly the achievement of complete reproductive isolation.

Given an adaptive infirmity of the hybrids (Vetukhiv, 1954a) and an opportunity for their formation (incipient sympatry), natural selection should, if possible, prevent their appearance. An adaptive inferiority of hybrids may, indeed, lower the fitness of both Mendelian populations between which such hybrids arise. Sexual (or psychological or ethological) isolation which makes the mutual attraction between conspecific males and females greater than the attraction between males and females of different species, is perhaps the most efficient means of accomplishing the prevention of gene exchange.

SUMMARY

Weak but statistically significant sexual isolation has been demonstrated among Vetukhiv's six experimental populations of *Drosophila pseudoobscura*, all originally descended from founders taken from cultures of the same hybrids from four geographic localities. These six populations were maintained separately for almost 4½ years and then tested for the existence of sexual isolation. The sexual isolation has arisen in the absence of any selection for isolation, evidently as a by-product of genetic divergence.

Professor Th. Dobzhansky supervised this entire project. Dr A. Mourad participated in many profitable discussions, and Mr G. Carmody kindly checked the mathematics in Table 1.

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